Can fast-growing plantation trees escape biochemical down-regulation of photosynthesis when grown throughout their complete production cycle in the open air under elevated carbon dioxide?

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ABSTRACT

Poplar trees sustain close to the predicted increase in leaf photosynthesis when grown under long-term elevated CO2 concentration ([CO2]). To investigate the mechanisms underlying this response, carbohydrate accumulation and protein expression were determined over four seasons of growth. No increase in the levels of soluble carbohydrates was observed in the young expanding or mature sun leaves of the three poplar genotypes during this period. However, substantial increases in starch levels were observed in the mature leaves of all three poplar genotypes grown in elevated [CO2]. Despite the very high starch levels, no changes in the expression of photosynthetic Calvin cycle proteins, or in the starch biosynthetic enzyme ADP-glucose pyrophosphorylase (AGPase), were observed. This suggested that no long-term photosynthetic acclimation to CO2 occurred in these plants. Our data indicate that poplar trees are able to ‘escape’ from long-term, acclimatory down-regulation of photosynthesis through a high capacity for starch synthesis and carbon export. These findings show that these poplar genotypes are well suited to the elevated CO2 conditions forecast for the middle of this century and may be particularly suited for planting for the long-term carbon sequestration into wood.

Key-words: ADP glucose phosphorylase; Calvin cycle enzymes; photosynthesis; Rubisco protein; starch.

INTRODUCTION

The CO2 concentration [CO2] in the Earth’s atmosphere is predicted to double over the next 50–100 years. Theoretically, this should result in a stimulation in photosynthetic carbon fixation of between 35 and 60%, primarily due to a reduction in photorespiration as the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation reaction is favoured in these conditions (Farquhar, von Caemmerer & Berry 1980; von Caemmerer & Farquhar 1981; Long 1991; Long et al. 2004). However, many plant species grown at elevated [CO2] exhibit an acclimatory down-regulation, decreasing photosynthetic potential, particularly with long-term growth in elevated [CO2] (Drake, Gonzalez-Meler & Long 1997; Long et al. 2004). This acclimatory response is often correlated with increased carbohydrate levels together with reductions in total nitrogen, Rubisco activity, Rubisco protein and/or Rubisco small subunit (rbcS) mRNA levels on a unit leaf-area basis (Rogers et al. 1998; Moore et al. 1999; Stitt & Krapp 1999; Rogers & Ellesworth 2002; Ainsworth & Long 2005). Increased levels of soluble sugars have been shown to down-regulate photosynthetic gene transcription, and it has been suggested that acclimation to elevated [CO2] may be mediated via hexose-cycling (Drake et al. 1997; Moore et al. 1998; Pego et al. 2000; Long et al. 2004). Although a correlation between carbohydrate accumulation and down-regulation of photosynthesis at elevated [CO2] has been observed in a number of studies, the extent and the nature of the acclimatory responses can vary depending on the species and growth conditions (Ainsworth et al. 2003 & Herrick & Thomas 2001). In addition, factors such as sink strength and nitrogen status have also been implicated in the acclimatory responses of plants grown at elevated [CO2] (Fischer et al. 1997; Ray & Jarvis 1998; Rogers et al. 1998; Heinke et al. 1999; Stitt & Krapp 1999; Isopp et al. 2000; Ainsworth et al. 2002; Long et al. 2004).

The photosynthetic rates of trees grown in elevated [CO2] have been shown to increase by up to 50% (Gunderson & Wullschleger 1994; Curtis 1996; Curtis & Wang 1998; Gunderson et al. 2002; Lee and Jarvis 1995; Pettersson & McDonald 1992; Long et al. 2004). These studies have focused on young trees or seedlings, which may exhibit a different response than mature trees, reaching canopy closure. A recent study of old-growth forest suggests that there
is no increase in carbon retention by the trees under elevated [CO$_2$] (Körner et al. 2005). If mature natural forests are unable to accumulate more C, then this leaves plantation forests as the only option for the partial mitigation of rising [CO$_2$] by woody systems (Eamus & Jarvis 1989). Fast-growing coppice poplar systems, producing repeated crops, of timber could be one system that would remove the CO$_2$ from the atmosphere at least over a period of decades, potentially slowing the rate of rise in atmospheric [CO$_2$] and buying time for longer-term solutions. The rapid growth of these trees also allows the analysis of the response to elevated [CO$_2$] over the complete production cycle, from planting through canopy closure and harvest. Recent analysis of photosynthetic capacity in three poplar clones grown under free-air CO$_2$ enrichment (EuroFACE) revealed, that in over three seasons, a significant and large increase in light-saturated photosynthesis (A$_{sat}$) was sustained (Bernacchi et al. 2003). However, these results also inferred that some transient acclimatory down-regulation of photosynthetic potential at elevated [CO$_2$] occurred. This acclimation was particularly evident in the season following coppice, when net photosynthesis was initially no higher with growth at elevated [CO$_2$] compared to growth at current ambient [CO$_2$] (Bernacchi et al. 2003). The lack of sustained and significant acclimation of photosynthetic potential, increased gross primary production and increased growth rates, at both leaf and canopy level, suggest that poplar trees may be able to escape long-term acclimatory changes that lead to more severe down-regulation of photosynthesis (Calfapietra et al. 2003; Taylor et al. 2003; Wittig et al. 2005). Although a number of studies have now been undertaken to investigate mature tree responses to long-term exposure to elevated [CO$_2$], much of this work has focused on gymnosperms grown for only part of their lifecycle at elevated [CO$_2$] (Liu et al. 2002; Rogers & Ellsworth 2002; Greenep et al. 2003; Ainsworth & Long 2005). There is little information on either carbohydrate status or the expression of photosynthetic proteins in closed-canopy stands of deciduous trees under long-term exposure to elevated [CO$_2$] in the open air (Ainsworth & Long 2005). Furthermore, little consideration has been given to the impact of elevated [CO$_2$] under field conditions on the responses of young sink leaves. The impact of elevated [CO$_2$] on leaves at different developmental stages has typically investigated short-term exposure in controlled environment conditions, with only one or two exceptions (Nie et al. 1995; Miller et al. 1997; Kauder, Ludewig & Heineke 2000). This study examined whether poplars, grown throughout a complete growth cycle in the field under elevated [CO$_2$], show evidence of biochemical acclimation in terms of accumulation of leaf carbohydrates and decreased expression of key enzymes of photosynthetic carbon metabolism.

**MATERIALS AND METHODS**

The EuroFACE facility, near Viterbo in Central Italy (Tuscany: 42°22′N, 11°48′E, altitude of 150 m), is located on a 9 ha field originally used for wheat cultivation and consists of a heavy loam soil. This entire field was planted with a poplar hybrid, *Populus x euramericana* Dode (Guinier) (*Populus deltoides* Bart. ex Marsh. × *Populus nigra* L., I-214) at 2 × 1 m spacing (5000 trees ha$^{-1}$). Within this field, six circular plots of 30 m diameter were established with three ‘control’ plots at current ambient [CO$_2$] (370 μmol mol$^{-1}$) and three more at elevated [CO$_2$] (550 μmol mol$^{-1}$). The construction and performance of this free-air concentration enrichment (FACE) system is described in detail by Miglietta et al. (2001). Each plot contained equal-sized segments planted with two poplar species, *Populus alba* L. (genotype 2AS11) and *P. nigra* L. (Jean Pourtet), and one interspecific hybrid *P. x euramericana*. Experimental plots were planted with cloned whips (sticks of about 20 cm) at 1 × 1 m spacing (10 000 trees ha$^{-1}$), resulting in approximately 350 trees per plot. A drip-irrigation system was installed to maintain soil moisture to prevent drought-stress. Further information on plant material and plantation layout is presented in Scarascia-Mugnozza et al. (2000) and Calfapietra et al. (2001). CO$_2$ fumigation began on 29 June 1999 just after planting, and was maintained from bud burst until leaf senescence of each year. The mean [CO$_2$] (± the standard deviation) was 544 ± 48, 532 ± 83, 554 ± 95 and 554 ± 87 mg g$^{-1}$ in 1999, 2000, 2001 and 2002, respectively (Calfapietra et al. 2001; F. Miglietta, CNR-IATA, Florence, Italy, unpublished results). The elevated [CO$_2$], averaged over 1 min intervals, were within ±20% of the pre-set target concentration for 89% of the time. The trees were coppiced between October 2001 and February 2002, and the experimental conditions were continued for re-growth during the 2002 and 2003 growing seasons.

**Photosynthesis in situ**

Photosynthetic gas-exchange measurements were conducted using portable open gas-exchange systems incorporating infrared CO$_2$ and water vapour analysers (LI-6400, LI-COR, Lincoln, NE, USA; CIRAS-1, PP systems, Hitchin, UK). To measure the diurnal response of leaf CO$_2$ uptake, gas exchange was measured from dawn to dusk at approximately 2.5 h intervals. Measurements were taken at the growth [CO$_2$] (370 or 550 μmol mol$^{-1}$) and at the temperature, vapour pressure deficit and quantum flux (Q) incident at that point in time. To maximize the accuracy of gas-exchange measurements, ‘steady-state’ rates of photosynthesis were measured. To achieve this, days with variable cloud cover, and therefore with frequent fluctuations in Q, were avoided. Additionally, leaves with a near-to-horizontal inclination were selected, and in an attempt to further minimize variation in Q, the leaf orientation and inclination remained unchanged during each photosynthetic measurement. Each individual measurement took approximately 30 to 60 s. The total daily photosynthetic carbon assimilation was calculated as the area under the curve describing the course of photosynthesis from dawn to dusk (Sigmaplot, SPSS Inc., Chicago, IL, USA).
Carbohydrate analysis

Samples were taken from the same leaves used for diurnal photosynthesis measurements and flash-frozen in liquid nitrogen, transported to the laboratory and stored at −80 °C. Water-soluble carbohydrates were extracted from the frozen leaf discs using a buffered ethanol extraction method and assayed for glucose, fructose and sucrose using the continuous NADP-linked, enzymatic substrate assay as described by Stitt et al. (1989), adapted for use with a microtitre plate reader (DIAS, Dynatech Laboratories Inc., Chantilly, VI, USA). To determine starch content, the insoluble leaf tissue remaining from the extraction of watersoluble carbohydrates was ground in liquid nitrogen and incubated with α-amylase (Sigma, St. Louis, MO, USA), and the glucose liberated from this reaction was determined using the continuous NADP-linked enzymatic assay (Stitt et al. 1989).

The total level of carbohydrate accumulating in the leaf over the photoperiod was calculated from the pre-dawn and dusk measurements of the soluble and insoluble sugars as described above. The daytime utilisation/export rates were obtained by subtracting the total carbohydrate accumulated during the photoperiod from the leaf CO₂ assimilation rate, integrated over the day and expressed as millimole glucose equivalents (Rogers et al. 2004).

Protein extraction and Western blotting

Samples were taken from the same leaves used for diurnal photosynthesis and carbohydrate measurements flash-frozen in liquid nitrogen, transported to the laboratory and stored at −80 °C. Leaf samples were ground in liquid nitrogen and the protein extracted as described previously (Harrison et al. 1998). The protein concentration in the samples was quantified and the samples loaded on an equal protein basis onto a 12% sodium dodecyl sulphate (SDS)–polyacrylamide gel. After separation was completed, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bradford, UK) and the resulting Western blot probed using antibodies raised against Rubisco large subunits [a gift from Dr. M. Parry of Institute of Arable Crops Research (IACR), Rothmasted, UK], sedoheptulose-1, 7-bisphosphatase (SBPase), fructose-1, 6-bisphosphatase (FBPase) and ADP-glucose pyrophosphorylase (AGPase) [a gift from Dr. P. Geigenberger of Max Planck Institut (MPI), Golm, Germany]. The proteins were detected using horseradish peroxidase-conjugated to the secondary antibody and enhanced chemiluminescence (ECL) detection reagent (Perbo Science Uk Ltd, Cramlington, Northumberland, UK), as described previously (Naidu et al. 2003). Samples were taken from a minimum of two trees from each of the six plots.

Statistical analysis

The significance of [CO₂] treatment upon carbohydrate accumulation and utilization was tested by an analysis of variance (ANOVA) (Systat, Evanston, IL, USA). To avoid pseudoreplication, the plots were treated as replicates rather than individual trees, giving a sample size of n = 3 per treatment.

RESULTS

Carbohydrate accumulation patterns

Leaf carbohydrate levels, sampled at the end of the photoperiod, were determined in three poplar genotypes grown in current ambient (unfilled bars) or elevated [CO₂] (filled bars) through four growing seasons (Fig. 1). In newly fully expanded mature sun leaves and over the two pre-coppice growth seasons (2000–2001) leading up to canopy closure and in the two post-coppice years, no increase in the levels of soluble carbohydrates was observed in any of the poplar clones in response to elevated [CO₂]. The only exception was in June 2001 when an increase in soluble sugars was evident in P. nigra (Fig. 1). In contrast to the soluble sugars, elevated [CO₂] caused substantial increases in starch by dusk in all three poplar clones on several days over the four growth seasons. In June 2000 and 2002, significant increases in starch levels were observed at the end of the day for all three Poplar clones (Fig. 2). However, in June 2001, only P. alba showed any increase in starch accumulation. In 2003, only P. x eurameriana was studied and samples for carbohydrate analysis were collected in June and August of that year. No [CO₂] treatment effect on the levels of soluble carbohydrates or starch was evident (Figs 1 & 2).

Daily carbohydrate accumulation and utilization

Carbon utilization patterns were determined in June and September 2000 and August 2003. This was achieved by subtracting the difference in pre-dawn and dusk total non-structural carbohydrate (TNC) and the calculated formation of carbohydrate from photosynthesis based on diurnal gas-exchange measurements. All three genotypes grown in elevated [CO₂] had higher levels of TNC at the end of the day compared to plants growing in ambient conditions in July and September 2000 (Fig. 3). The picture for pre-dawn carbohydrates is less consistent. In September 2000 and August 2003, no difference in accumulation of TNC was evident between the trees in elevated or ambient [CO₂]. In contrast, in July 2000, pre-dawn carbohydrate levels more than doubled in the leaves of plants grown in elevated [CO₂]. At this time of day (July 2000), the amount of TNC accumulated was small relative to the total carbohydrate accumulated by the end of the day, independent of [CO₂]. The diurnal course of photosynthesis showed that on 23 August 2003, a 46% (P < 0.01) increase in daily photosynthesis was evident in the most recently fully expanded leaves of P. x eurameriana (Table 1). This result is in keeping with our previous finding in the 2000 season when increases in the photosynthesis of P. x eurameriana were in the range of 44 to 47% (P < 0.001) (Table 1). Using these data, the daily integral of photosynthesis was used to calculate the hexose equivalents fixed in
the photoperiod, as described previously for soybean (Rogers et al. 2004). This showed that in elevated [CO2], all three genotypes on all three dates produced over 50% more hexose equivalents during the day than those plants grown in ambient conditions. Furthermore, most (>90%) of the carbon fixed within a day was exported or utilized before dusk, and the daytime export/utilization rates were higher in elevated [CO2] when compared to current ambient [CO2] for all of the genotypes (Fig. 3).

Carbohydrate accumulation patterns were also determined in young expanding leaves (LPI 3) stage as described by Taylor et al. (2003). In these leaves, no differences were found in the levels of pre-dawn carbohydrates in elevated [CO2] and only a small, but insignificant, increase was evident at the end of the day when compared to equivalent leaves in ambient [CO2] (Fig. 4).

**Chloroplast protein expression**

Western blot analysis was used to determine the impact of growth in elevated [CO2] on the levels of photosynthetic proteins. Proteins were prepared from discs sampled from a parallel sample of leaves to those used for carbohydrate determination. The data shown in Figs 5 and 6 is for samples

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**Figure 1.** Soluble carbohydrate levels in three poplar genotypes grown in current ambient (unfilled bars) or elevated CO2 concentration [CO2] (filled bars) through four growing seasons. The leaves were sampled at the end of the photoperiod. [CO2] were 370 and 550 μmol mol⁻¹ for the control and elevated plots, respectively. Data represents the mean of three replicate plots with a minimum of two subsamples per plot. Error bars represent ±1 standard error of the mean. The significance of [CO2] upon carbohydrate content was tested by an analysis of variance (ANOVA). Significant differences between [CO2] treatments are indicated as follows: ***P < 0.01, **P < 0.05 and *P < 0.10.

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**Table 1.** Total daily photosynthetic carbon assimilation (mmol m⁻² photoperiod⁻¹) in three poplar genotypes grown in current ambient (control) or elevated [CO2] during two growing seasons

<table>
<thead>
<tr>
<th>Clone</th>
<th>July 2000</th>
<th>September 2000</th>
<th>August 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Populus alba</td>
<td>462 ± 24</td>
<td>774.7 ± 35.0</td>
<td>696 ± 19</td>
</tr>
<tr>
<td>Populus x euramericana</td>
<td>816 ± 66</td>
<td>1171 ± 38</td>
<td>722 ± 15</td>
</tr>
<tr>
<td>Populus nigra</td>
<td>725 ± 10</td>
<td>1045 ± 4</td>
<td>651 ± 48</td>
</tr>
</tbody>
</table>

Data represents the mean and ±1 standard error of the mean of at least two replicate plots. [CO2], CO2 concentration.
loaded on an equal protein basis, but it should be noted that the same results were obtained when proteins were loaded on the basis of equal leaf area. No detectable [CO₂] treatment effect on Rubisco, FBPase or SBPase protein levels were evident in any of the poplar genotypes under elevated [CO₂] at any sampling point (Fig. 5a). Given that starch levels were increased in all three poplar clones, at several time points throughout this experiment, Western blotting was extended and the levels of the small subunit of the starch biosynthetic enzyme AGPase were examined. No change in the level of this protein was detected (Fig. 5b).

Western blot analysis of proteins taken from LPI 3 showed that the levels of Rubisco, SBPase and FBPase per unit area were lower when compared to mature leaves in both ambient and elevated [CO₂]. However, there was no detectable difference in the levels of photosynthetic proteins in the young leaves in ambient [CO₂] compared to equivalent leaves in elevated [CO₂] (Fig. 6).

**DISCUSSION**

The accumulation of carbohydrate in leaves has frequently been associated with a reduction in the levels of Rubisco protein leading to a down-regulation in maximum photosynthetic capacity in plants grown in elevated [CO₂] (Fischer et al. 1997; Ray & Jarvis 1998; Rogers et al. 1998; Stitt & Krapp 1999; Ainsworth et al. 2002; Long et al. 2004). In this experiment, the level of soluble carbohydrates accumulated over the photoperiod, in three poplar genotypes, was not increased significantly over the three diurnal courses investigated, in either young or fully expanded leaves. In contrast, large increases in starch levels were observed at least once in each of the years studied, in fully expanded leaves. These occasions represented all stages in the growth cycle, the exponential growth phase, canopy closure, maturation prior to harvest and re-growth following harvest. No change in the levels of Rubisco protein or that of the two additional Calvin cycle enzymes FBPase and SBPase – both key metabolic control points, was evident. This provides clear evidence that the long-term acclimatory changes typical of the tree systems previously studied (Ainsworth & Long 2005) are not inevitable and was not found in these clones selected for high productivity. In the season immediately following coppice when starch accumulation was high in all three poplar clones, acclimatory down-regulation of photosynthetic capacity – leading to a loss of stimulation of A_sat, was transiently evident (Bernacchi et al. 2003). However, even in this case, no...
A decrease in the levels of photosynthetic proteins was seen. These results suggest that the intermittent down-regulation in photosynthetic capacity observed in the poplar genotypes studied here was not due to the repression of photosynthetic protein expression. Given that the observed reductions in the maximum potential photosynthetic capacity were small (less than 10%), it is likely that short-term feedback responses were occurring at the level of enzyme activity in these poplar trees.

In a number of different experiments, starch accumulation has been shown to increase in plants grown in elevated [CO$_2$], and in these examples, no acclimatory responses were reported suggesting that there is no direct correlation between high levels of starch accumulation and photosynthetic acclimation (Moore et al. 1998; Rogers et al. 1998; 2004; Ainsworth et al. 2002). It is possible that the increased starch levels observed in the poplar trees grown in elevated [CO$_2$] rather than feeding back to reduce photosynthetic capacity, enabled these plants to avoid the ‘down-regulation’ of photosynthesis by preventing cycling and/or accumulation of soluble sugars. Further support for this hypothesis is provided from work on mutants with altered starch synthesis ability, where there is a correlation between down-regulation of photosynthetic gene expression in response to elevated [CO$_2$] and the levels of mRNA encoding the starch biosynthetic enzyme AGPase (Ludewig et al. 1998; Heineke et al. 1999). To examine the possibility that the stimulation of the starch biosynthetic capacity may

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**Figure 3.** Carbon utilization at dawn and dusk in June and September 2000 and August 2003. Total carbohydrate concentration pre-dawn and end of day, carbohydrate accumulation, carbohydrate produced in the photoperiod determined from diurnal photosynthesis measurements, daytime carbohydrate export and nighttime export or respiratory loss. Treatments and statistics are the same as for Fig. 1.

<table>
<thead>
<tr>
<th>Populus species</th>
<th>July 2000</th>
<th>September 2000</th>
<th>August 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dawn TNC</td>
<td>CO$_2$***</td>
<td>CO$_2$**</td>
<td>CO$_2$**</td>
</tr>
<tr>
<td>End of day TNC</td>
<td>CO$_2$***</td>
<td>CO$_2$**</td>
<td>CO$_2$**</td>
</tr>
<tr>
<td>TNC accumulated in photoperiod</td>
<td>CO$_2$***</td>
<td>CO$_2$**</td>
<td>CO$_2$**</td>
</tr>
<tr>
<td>Hexose equivalents produced in photoperiod</td>
<td>CO$_2$***</td>
<td>CO$_2$**</td>
<td>CO$_2$**</td>
</tr>
<tr>
<td>Day time utilization/export</td>
<td>CO$_2$***</td>
<td>CO$_2$**</td>
<td>CO$_2$**</td>
</tr>
<tr>
<td>Apparent night time export</td>
<td>CO$_2$**</td>
<td>CO$_2$*</td>
<td></td>
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</table>
involve changes at the level of gene expression, we have analysed the levels of AGPase protein in *P. x euramericana*. No detectable increase in the level of AGPase protein was evident. However, a new post-translational activation mechanism involving redox changes had been shown to modulate leaf AGPase activity, and it is conceivable that this is the mechanism responsible for the increased starch synthesis in poplar trees in elevated [CO₂] (Hendriks *et al.* 2003).

Diurnal photosynthesis in poplar trees grown in elevated [CO₂] over four growing seasons showed a sustained increase in photosynthesis of between 35 and 60% prior to coppicing (Bernacchi *et al.* 2003). Here we show that this increase in daily photosynthesis is maintained during the re-growth following coppicing in *P. x euramericana*, which produced the most biomass at coppice (Calfapietra *et al.* 2003). Parallel analysis of diurnal carbohydrate accumulation patterns indicated that although more starch accumulated in these leaves during the day, increased export and

![Figure 4](image1.png)

**Figure 4.** The daily pattern of carbon accumulation in August 2003 in a young expanding leaf (LPI 3). Total carbohydrate concentration pre-dawn and end of day, and carbohydrate accumulation. Treatments and statistics are the same as for Fig. 1. TNC, total non-structural carbohydrate.

![Figure 5](image2.png)

**Figure 5.** Western blot analysis of protein samples from *Populus x euramericana* and *Populus nigra* grown in current ambient or elevated CO₂ concentration [CO₂] through three growing seasons. Protein prepared from leaf samples were taken at the same time and from the same leaves used for carbohydrate analysis (Figs 1 & 2). The proteins (25 μg) were loaded in each lane, separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and blotted onto nitrocellulose. The resulting blots were probed with polyclonal antibodies: (a) large subunit ribulose 1,5-bisphosphate carboxylase/oxygenase (LSU Rubisco), fructose-1, 6-biphosphatase (FBPase), sedoheptulose-1, 7-biphosphatase (SBPase) for *P. x euramericana* and (b) ADP-glucose pyrophosphorylase (AGPase) for *P. x euramericana* and *P. nigra*. E, Elevated; C, Control.
Western blot analysis of protein samples from young expanding leaves (LPI 3) of *Populus x euramericana* grown in current ambient or elevated CO₂ concentration [CO₂] in 2003. Leaf samples were taken at the same time and from the same leaves used for carbohydrate analysis (Fig. 4). The proteins (25 μg) were loaded in each lane, separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and blotted onto nitrocellulose. The resulting blots were probed with polyclonal antibodies: large subunit ribulose 1,5-bisphosphate carboxylase/oxygenase (LSU Rubisco), fructose-1, 6-bisphosphatase (FBPase) and sedoheptulose-1, 7-biophosphatase (SBPase). ADP-glucose pyrophosphorylase (AGPase); E, Elevated; C, Control.

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